NTP
National Toxicology Program

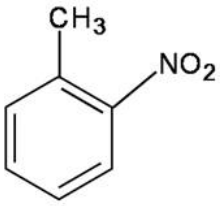
Report on Carcinogens
Draft Substance Profile on *o*-Nitrotoluene

Gloria D. Jahnke, DVM
National Institute of Environmental Health Sciences
NTP Board of Scientific Counselors Meeting
Research Triangle Park, NC
February 24, 2009

NTP
National Toxicology Program

o-Nitrotoluene

CAS No. 88-72-2



2-nitrotoluene; 2-Methyl-1-nitrobenzene; 2-methylnitrobenzene; o-nitrophenylmethane; 2-nitrotoluene; 2-nitrotoluol; alpha-methylnitrobenzene; 1-methyl-2-nitro-benzene; methylnitrobenzene; ONT

Objectives

To present the science that supports the preliminary listing recommendation for *ortho*-nitrotoluene in the 12th RoC as *Reasonably Anticipated to be a Human Carcinogen*

- Information on use and exposure in US
- Cancer studies in humans and experimental animals
- Mechanistic evidence that supports the recommendation

Uses

- *o*-Nitrotoluene (*o*-NT) is a chemical intermediate used in the synthesis of azo dyes, magenta dyes, and sulfur dyes.

- It is also used (either directly or as an intermediate) in the production of explosives, agricultural chemicals, pesticides, petrochemicals, pharmaceuticals, and rubber products.

Significant U.S. Exposure

- High production volume chemical; in US, 10-50 million pounds per year (2002)
- Occupational exposure during chemical production and use as an intermediate; detected in workplace air
- Detected in groundwater, surface water, and soil at or near munitions production and military training facilities

Human Cancer Studies

- Data from the studies inadequate to evaluate the relationship between human carcinogenicity and exposure specifically to *o*-NT
- Three studies of magenta manufacturing workers
  - Findings of excess risk of bladder cancer
  - Only one study noted *o*-NT exposure; however, workers also exposed to other aromatic hydrocarbons

Sufficient Evidence from Studies in Experimental Animals

Early Onset of Tumors

NTP subchronic feed studies in rats (F344/N) and mice ($B6C3F_1$)

13-wk exposure (both sexes)

- mesothelioma of tunica vaginalis of testis in rats

26-wk exposure or 13-wk exposure & 13-wk recovery period (male rats only)

- mesothelioma of tunica vaginalis of testis & cholangiocarcinoma of the liver in male rats

Sufficient Evidence from Studies in Experimental Animals

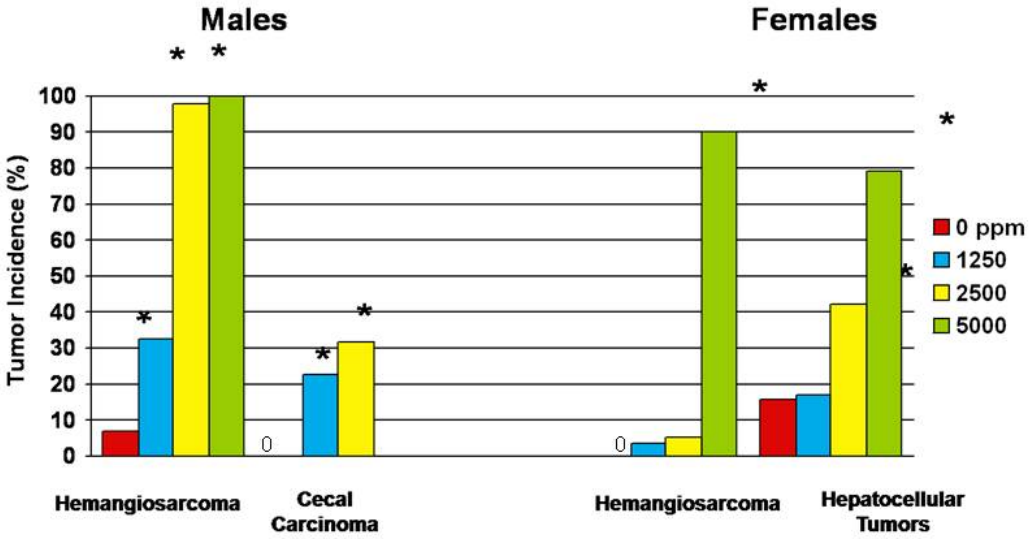
Tumors at multiple sites

NTP chronic feed studies in rats (F344/N) and mice ($B6C3F_1$)

- Two-year exposure (both sexes)
- 13-wk exposure & recovery period to two years (male rats only)

NTP conclusion: ***clear evidence of carcinogenicity***
in rats and mice for both sexes

Tumors in Mice Following Dietary Exposure to *o*-Nitrotoluene For Two Years



- Dose-dependent responses
- Tumors in males and females at multiple sites

Survival-Adjusted incidence, *$P<0.001$

Tumors in Rats Following Dietary Exposure to *o*-Nitrotoluene for Up to Two Years

Tissue	Males		Females
	Chronic	13 wk Exposure	Chronic
Malignant Mesothelioma	+	+	–
Mammary Gland Fibroadenoma	+	+	+
Subcutaneous			
Lipoma	+	+	–
Fibroma/Fibrosarcoma	+	+	+
Hepatocellular Tumors	+	+	+
Cholangiocarcinoma	–	+	–
Lung Tumors	–	+	–

Tumors at multiple sites in males and females

Mechanistic Evidence

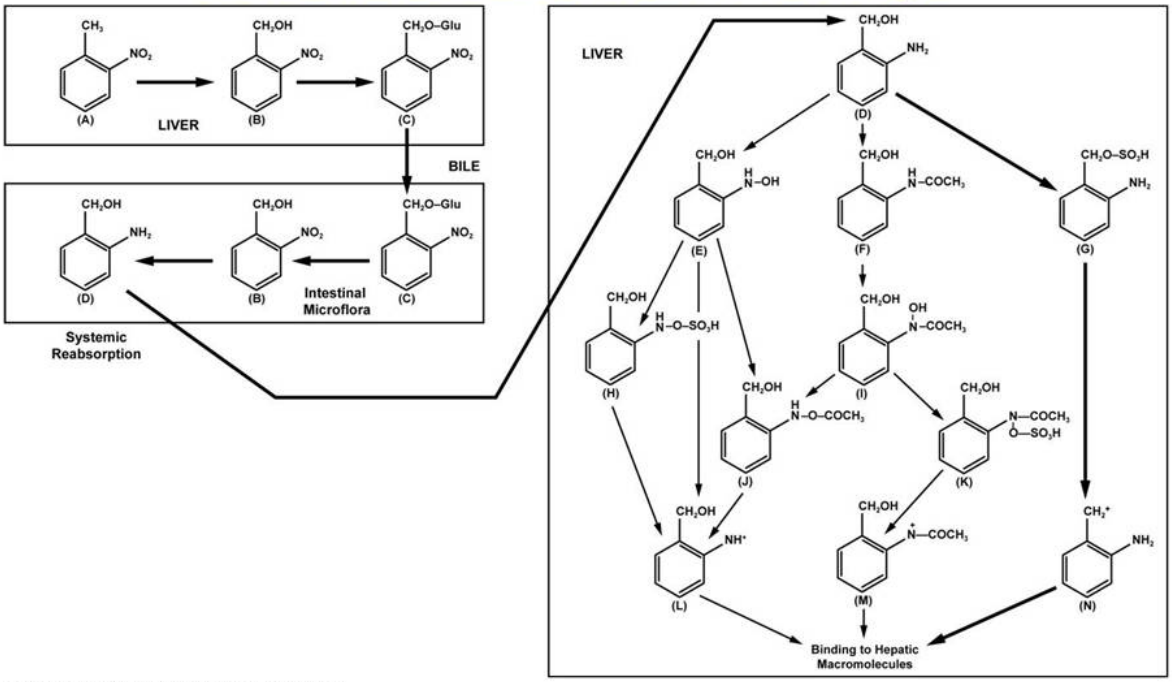
- Urinary metabolites
- Proposed bioactivation pathways
- Evidence for other proposed mechanisms

Urinary Metabolites

Metabolite	Humans	Rats	Mice
o-Nitrobenzoic Acid	+	+	+
o-Nitrobenzyl Alcohol	+	+	–
o-Nitrobenzylglucuronide	NR	+	+
o-Aminobenzyl Alcohol	NR	+	–

NR=Not Reported
– = Metabolite not found

Potential Bioactivation Pathways



Adapted from Chism & Rickert (1985)

Supporting Mechanistic Data

- Intestinal bacteria necessary for bioactivation
  - Did not induce repair in human or rat hepatocytes *in vitro*
  - DNA adducts and increased repair in liver of male rats, but not germ-free male rats
- Hepatic DNA adducts increased with *o*-NT dose

Supporting Mechanistic Data

Carbonium and nitrenium ions of 2-methylaniline form hemoglobin adducts and DNA adducts

- 2-Methylaniline hemoglobin adducts and DNA adducts identified in rats exposed to *o*-NT
- Hemoglobin adduct levels proportional to DNA adduct levels in the liver

Evidence that human exposure results in production of reactive metabolites

- 2-Methylaniline hemoglobin adducts detected in workers

Other Mechanisms of Carcinogenesis

- Tumors found in both sexes at multiple sites in rodent studies
  - Neither *o*-aminobenzyl alcohol nor its metabolites detected in mouse urine after *o*-NT exposure
- Mutations in *p53*, *beta-catenin*, *K-ras* genes in *o*-NT induced hemangiosarcomas and colon tumors (mice)
  - *p53* and *K-ras* mutations consistent with targeting of guanine DNA adduct formation

Proposed *o*-Nitrotoluene Listing

o-Nitrotoluene *is reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting mechanistic data.